PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : G01N 33/573, 33/552, C12Q 1/68

A1

(11) International Publication Number: WO 91/08489

(43) International Publication Date: 13 June 1991 (13.06.91)

(21) International Application Number: PCT/US90/06646

(22) International Filing Date: 14 November 1990 (14.11.90)

(30) Priority data:
444,297 1 December 1989 (01.12.89) US

(71) Applicant: PACKARD INSTRUMENT COMPANY, INC. [US/US]; 2200 Warrenville Road, Downers Grove, IL 60515 (US).

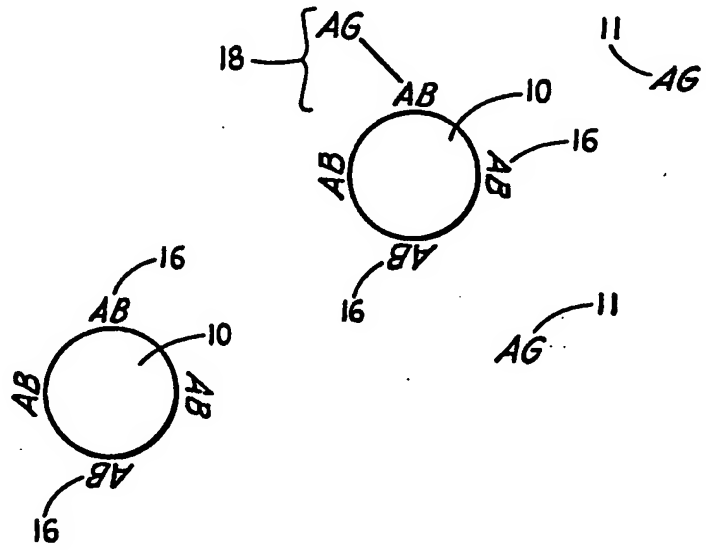
(72) Inventors: VAN CAUTER, Gustaaf, C. ; 5203 Tonnbrooke, Middletown, CT 06457 (US). OSTEN, Donald, E. ; 453 Nassau Avenue, Bolingbrook, IL 60439 (US). NEUMANN, Kenneth, E. ; 8439 Roseview Drive, Niles, IL 60648 (US).

(74) Agent: RUDISILL, Stephen, G.; Arnold, White & Durkee, P.O. Box 4433, Houston, TX 77210 (US).

(81) Designated States: AT (European patent), BE (European patent), CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), LU (European patent), NL (European patent), SE (European patent).

Published
With international search report.

(54) Title: SCINTILLATION PROXIMITY RADIOIMMUNOASSAY USING SOLID SCINTILLATOR SUPPORT BODY



(57) Abstract

A new support body (10) for use in a scintillation proximity radioimmunoassay consisting of a scintillation material. The support body (10) of the present invention permits extremely sensitive and specific assays for a broad range of biological and non-biological substances. The support body (10) of the present invention having a ligand (16) coupled to its surface which is capable of selectively biochemically binding to a reactant of interest (11) resulting in a complex (18).

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	**FI**	Finland	**ML**	Mali
AU	Australia	**FR**	France	**MN**	Mongolia
BB	Barbados	**GA**	Gabon	**MR**	Mauritania
BE	Belgium	**GB**	United Kingdom	**MW**	Malawi
BF	Burkina Faso	**GN**	Guinea	**NL**	Netherlands
BG	Bulgaria	**GR**	Greece	**NO**	Norway
BJ	Benin	**HU**	Hungary	**PL**	Poland
BR	Brazil	**IT**	Italy	**RO**	Romania
CA	Canada	**JP**	Japan	**SD**	Sudan
CF	Central African Republic	**KP**	Democratic People's Republic of Korea	**SE**	Sweden
CG	Congo			**SN**	Senegal
CH	Switzerland	**KR**	Republic of Korea	**SU**	Soviet Union
CI	Côte d'Ivoire	**LI**	Liechtenstein	**TD**	Chad
CM	Cameroon	**LK**	Sri Lanka	**TG**	Togo
DE	Germany	**LU**	Luxembourg	**US**	United States of America
DK	Denmark	**MC**	Monaco		
ES	Spain	**MG**	Madagascar		

SCINTILLATION PROXIMITY RADIOIMMUNOASSAY USING SOLID SCINTILLATOR SUPPORT BODY

Background Of The Invention

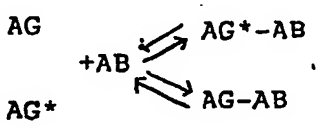
1. Field Of The Invention

The present invention relates to scintillation proximity radioimmunoassay, and more particularly to compositions and methods for improving the sensitivity of scintillation proximity radioimmunoassay.

2. Description of the Related Art

Because of its specificity, sensitivity, and technical simplicity, radioimmunoassay has found increased use in recent years in clinical, research, and industrial laboratories. Radioimmunoassay is primarily useful in detecting and quantifying picogram quantities of a reactant of interest. This reactant may be a ribonucleic acid, a deoxyribonucleic acid, an antibody, an antigen, a metabolite, a biological receptor antagonist, a biological receptor agonist, a drug, a hormone, or a vitamin. The detection and quantification of the above reactants can be useful in the diagnosis of several clinical conditions, i.e., pregnancy, hepatitis, genetic abnormality, or infection.

The basic principle of radioimmunoassay is shown in the following reaction:



According to the reaction set forth above, the presence of the reactant of interest (AG) is detected and quantified by a

reaction in which radiolabelled reactant (AG*) and non-labelled reactant (AG) compete to biochemically bind to a ligand (AB). For the purposes of this invention, the term ligand or (AB) is used to describe any molecule which will selectively and specifically biochemically bind to another specific and distinct molecule identified for the purposes of this invention as the reactant of interest or (AG). In order to perform a competitive or direct assay, AG must also be available in a radiolabelled form, e.g., AG*. In radioimmunoassay ^{125}I, ^{14}C and ^{3}H are the most frequently used radiolabels, with ^{32}P, ^{99m}Tc and ^{57}Co also finding limited use.

According to a well known protocol for competitive radioimmunoassay, a known quantity of AG* and an unknown quantity of AG are added to an appropriate aqueous medium containing AB. AB, thereafter, binds indiscriminately with AG or AG* to form complexes AG-AB and AG*-AB. AG and AG* bind with AB in proportion to their relative concentrations in the sample. After an incubation period, wherein the reaction has reached equilibrium, the unbound reactants, free-AG and free-AG*, are separated from the complexed reactants, AG-AB and AG*-AB. Separation is necessary since it is the concentration of AG*-AB which is indicative of the concentration of AG. The concentration of AG*-AB is determined by measuring the amount of radioactive energy emitted by the complexed reactants after all free-AG* has been removed.

Separation is accomplished by either absorption or precipitation, or by utilizing ligands bound to a solid-support structure. In absorption, the complexed reactants are

absorbed onto activated charcoal, dextran-coated charcoal, talc, etc. The absorbing material is thereafter removed from the medium, typically by centrifugation, and washed several times to remove any excess free-AG*. In precipitation, the complexed reactants are precipitated from solution by a protein precipitator such as $(NH_4)_2SO_4$, ethanol dioxane, or polypropylene glycol. The precipitate is then separated and washed to remove excess free-AG*. When utilizing ligands attached to a solid-support structure, the solid-support is withdrawn from the sample and washed to remove excess free-AG*.

The radioactivities of the remaining aqueous sample and the separated complexed reactants are thereafter measured and compared to determine the concentration of AG. For example, a high concentration of AG in the sample will result in a high concentration of AG-AB, and accordingly, a low concentration of AG*-AB. Therefore, the overall radioactivity of the separated complexed reactants is low. Thus, the radioactivity emitted by the separated complexed reactants is inversely proportional to the amount of AG in the sample being tested. By developing standard curves very accurate measurements of AG are possible.

However, a major drawback to this type of radioimmunoassay is that the procedure requires the separation of the bound and unbound reactants. As set forth above, the separation procedures require a significant number of time-consuming and expensive steps. For example, each of the separation procedures involves repeatedly washing the

separated complexed reactants prior to measuring radioactivity of the fractions. This not only is time consuming, but it also produces volumes of radioactive waste, which is difficult to handle and expensive to dispose of.

Scintillation proximity radioimmunoassay (SPRA) was recently developed to overcome the problems associated with prior radioimmunoassay techniques while maintaining specificity. SPRA combines the techniques of scintillation counting and radioimmunoassay. Scintillation counting is a generally known and widely used technique for the measurement of low energy beta emitting radionuclides in addition to gamma and alpha emitters. Scintillation counting is utilized to make quantitative measurements of radioactivity by incorporating a radiolabelled analyte into a solution with a scintillator capable of producing photons resulting from the kinetic interaction of nuclear decay products. For example, a tritium-labeled compound is incorporated in a solution containing a scintillator, energy from the beta decay is transferred with reasonable efficiency to the scintillator upon kinetic interaction. This transferred energy excites the scintillator which thereafter emits multiple photons of light proportional to the energy of the beta particle. The light energy is detected by sensitive photomultiplier tubes of a scintillator counter. Chemically, SPRA is similar to other radioimmunoassays, relying on the biochemical binding of radiolabelled reactant AG* and non-radiolabelled reactant AG to a ligand AB. However, instead of separating the bound from the unbound reactants, SPRA takes advantage of the relatively

weak radioactive energies emitted by the radiolabel to accurately and instantaneously measure the amount of complexed radiolabelled reactant, from which the amount or presence of the non-radiolabelled reactant can be extrapolated.

The basic principle of SPRA is shown in FIG. 3. As shown in FIG. 3, SPRA generally, in a competitive assay, utilizes AG, AG*, AB and microscopic support bodies. Typically, these support bodies are formed as amorphous bodies, referred to as beads. Attached to the surface of these beads are ligands AB. The ligands are typically bound to the surface of the bead covalently. The beads also contain a suitable scintillator integrated within the body of the bead.

The radiolabels commonly used in radioimmunoassay emit relatively weak energies. For example, beta particles emitted during ^{3}H decay have a range of only a few microns in water. Therefore, in order for a radiolabeled compound to be detected by the scintillation counter, the radiolabels must be brought into close enough proximity to the bead to excite the scintillator within. In a dilute suspension of beads and radiolabeled compounds, few of the radiolabels would be in a close enough proximity to a bead to excite the scintillator, and thus one could predict that very few of the radiolabelled compounds would be detected. However, because the beads have attached to their surface AB, AG* readily attaches to the surface of the bead. This reaction brings the radiolabel of AG* into close enough proximity to the scintillator to excite it. Therefore, the number of radiolabeled compounds detected markedly increases. These results are expressed as levels of

radioactivity in the sample as measured by a scintillation counting system.

Thus, according to well established SPRA protocol, a known amount of AG* and an unknown amount of AG are added to an appropriate aqueous suspension containing a plurality of microscopic support bodies, each support body having bound to its surface a multiplicity of AB molecules. AG and AG* indiscriminately and competitively biochemically bind to AB thus bringing the radiolabel of AG* into close enough proximity to the bead to allow the radiation emitted by the radiolabel to excite the scintillator within the bead. When excited, the scintillator emits detectable photons of light, which, in turn, are detected by a liquid scintillation counting system. The detected photons are utilized by the liquid scintillation counting system to calculate a level of radioactivity for the sample being assayed. After an appropriate incubation period, wherein the reaction reaches equilibrium, the radioactivity level of the sample is measured by the liquid scintillation counting system, and the resulting measurement is used to determine concentration of AG in the sample. For most assays a standard curve is developed (radioactivity detected vs. concentration of AG) wherein a high level of radioactivity detected corresponds to a low concentration of AG. Accordingly, in a competitive SPRA the level of detected radioactivity is inversely proportional to the concentration of AG in the sample being tested. Thus, by using SPRA the concentration of AG may be continuously and

sensitively monitored without the need for a separation or washing procedure.

However, a drawback in SPRA are the support bodies utilized in conducting the assay. The support bodies presently utilized in SPRA are complicated structures which are expensive to manufacture. Moreover, these support bodies inefficiently detect the presence of AG*-AB complexes bound to their surface.

According to European patent application No. 154,734, support bodies useful in SPRA are constructed by first forming a porous amorphous microscopic particle, typically referred to as a bead. These particles are typically constructed from acrylamide, acrylic acid, polymers of styrene, agar, agarose, polycarbonate, polypropylene, polystyrene, cellulose acetate or latex. Thereafter, cyanogen bromide is incorporated into the bead for the effective binding of ligands to the surface.

In order for these materials to be useful in SPRA a scintillating material must be incorporated within their structure. This is accomplished by first choosing a water insoluble scintillator. A solvent is then chosen which is miscible with both water and the scintillator; for instance, dimethyl sulfoxide (DSMO) is an effective solvent if diphenyloxazole (PPO) is utilized as the scintillator. The beads are then placed into the solvent containing an appropriate concentration of the scintillator. The scintillator and solvent are allowed to permeate throughout the body of the porous beads, and the beads are thereafter removed and placed in water, precipitating the scintillator

within the body of the bead. The beads are removed from the water and washed to remove any excess solvent and precipitated scintillator from their surface. The beads are then suspended in a solution containing both a detergent, such as Tween 20, to prevent the beads from sticking, and gelatin to bind any sites on the surface of the porous bead not bound to a ligand. This lowers the non-specific binding of the beads. Finally, a bactericide, such as sodium azide, is applied to the beads to prevent bacteria growth thereon.

As is apparent to those skilled in this art, the above method for producing support bodies requires several time-consuming operations that are often complicated and expensive. Further, the finished product, a bead randomly incorporating a scintillator throughout its body, is only partially effective as a means for detecting the proximity of a radiolabel. Beads presently used in SPRA, as described in the referenced European patent application, only randomly scatter scintillator throughout their entirety. Thus, the amount and distribution of scintillator in each bead is variable, and accordingly, no two beads will respond similarly when in close proximity to a radiolabel. For example, in beads with less scintillator, particulate energized matter, such as a beta particle or auger electron, will, in several instances, pass through the body of the bead without impacting and exciting a scintillator. Thus, a significant number of radioactive events will not be detected and the assay results will be flawed. Further, even in beads containing a large quantity of

scintillator this "pass through" effect will occur since the beads have scintillator just randomly scattered throughout.

Thus, it is an object of the present invention to address one or more of the foregoing deficiencies in the art by providing an improved method for SPRA.

A particular object of the present invention is to provide a more accurate, less costly method for conducting SPRA which produces more reproducible results.

A more particular and highly important object of the present invention is to provide an inexpensive support body for use in SPRA which can be mass produced to give reproducible and more accurate results, which until now have been impossible to obtain.

Summary of the Invention

The foregoing objects are achieved with the present invention by providing a reagent for use in SPRA consisting of a support body constructed from scintillating material, the support body having coupled to its surface a multiplicity of ligands. The ligands are capable of selectively biochemically binding to a reactant of interest.

Another aspect of the present invention is directed to the process of assaying an aqueous suspension containing a known concentration of a radiolabelled reactant and an unknown concentration of a non-radiolabelled reactant. The reactant is selected from the group consisting of antibodies, antigens, deoxyribonucleic acids, ribonucleic acids, biological receptors, biological receptor antagonists, biological

receptor agonists, steroids, enzymes and vitamins. The inventive process includes several steps. One step in the inventive method is adding to an aqueous suspension a plurality of scintillating support bodies consisting of a scintillating material and having coupled to their surfaces a multiplicity of ligands. The ligands are capable of selectively biochemically binding to the reactant, regardless of the presence of a radiolabel. The radiolabeled reactant emits radiation energy capable of exciting a scintillating support body upon biochemically binding to a ligand. The scintillating support body emits detectable photons of light when excited. Another step in the inventive process is detecting the photons of light emitted by the plurality of scintillating support bodies in the aqueous medium. The photons of light detected are indicative of the concentration of the non-radiolabeled reactant.

A further aspect and a preferred embodiment of the present invention is directed to a process for assaying an aqueous sample to determine the concentration of a reactant of interest using scintillation proximity radioimmunoassay. A first step in the inventive process is providing a plurality of scintillating support bodies consisting of yttrium silicate and from about 0.1 to about 10.0% by weight of an inorganic salt of cerium. A multiplicity of ligands are then coupled to the surface of the support bodies. The ligands are capable of selectively biochemically binding a reactant of interest, regardless of whether it is radiolabelled. A known quantity of the reactant is chemically coupled to a radiolabel, and is

thereafter mixed in an aqueous suspension with an unknown quantity of non-radiolabelled reactant and a plurality of support bodies. Both radiolabelled and non-radiolabelled reactants compete to biochemically bind to the ligands coupled to the surface of the support body. The radiolabelled reactant emits radiation energy capable of exciting the scintillating support body upon biochemically binding to a ligand. The scintillating support body emits detectable photons of light when excited. The photons of light emitted by the plurality of scintillating support bodies are detected. The photons of light detected are indicative of the concentration of the non-radiolabeled reactant.

The support bodies, reagents, and assay processes of the present invention may be utilized in conjunction with any ligand-reactant combination that specifically biochemically binds together and in which the reactant may be radiolabelled without effecting its specificity for the ligand. Ligand-reactant combinations with which the present invention is especially useful include: (1) receptor-agonist; (2) receptor-antagonist; (3) enzyme-substrate or co-factor; (4) RNA-DNA; and (5) DNA-DNA. It is understood that in the present invention either element may serve as a ligand or a reactant.

Brief Description Of The Drawings

FIG. 1 is a schematic representation of the specific biochemical binding of the reactant of interest (AG) to a ligand (AB) which, in turn, is coupled to the surface of a scintillating support body, represented as a sphere;

FIG. 2 is a schematic representation of a direct scintillation proximity radioimmunoassay, wherein the radiolabeled reactant of interest (AG*) binds to a ligand (AB), thus bringing the radiolabel of AG* into a close enough proximity to the scintillating support body, represented as a sphere, to allow the emitted radiation of the radiolabel to excite the scintillating support body, thereby causing the scintillating support body to emit photons of light which are detected by a detector;

FIG. 3 is a schematic representation of a competitive scintillation proximity radioimmunoassay, wherein the non-radiolabeled reactant (AG) and the radiolabeled reactant (AG*) compete to biochemically bind to the ligand (AB) which is coupled to the surface of the scintillating support body, represented as a sphere, AG* upon binding to AB brings the radiolabel of AG* into a close enough proximity to the scintillating support body to allow the radiolabel to excite the scintillating support body, thereby causing the scintillating support body to emit photons of light which are detected by a detector; and

FIG. 4 is a graphic representation of trials conducted to determine the counting efficiencies of scintillating support bodies having diameters of 3, 10 and 100 microns.

Description of the Preferred Embodiments

In accompanying FIGS. 1, 2, and 3, scintillating support bodies 10, 100 and 200 are represented as suspended in an aqueous solution. The preferred suspending agent is glycerol.

The scintillating support bodies consist of a scintillating material. Because SPRA is performed in an aqueous medium, the preferred scintillating material must be insoluble in water. The preferred scintillating materials of the present invention are base glasses which when appropriately activated or doped respond as scintillators and emit detectable photons of light when excited by the kinetic interaction of nuclear decay particles, or calcium fluoride. The preferable activating materials or dopants are selected from the group comprising Mn, Cu, Pb, Sn, Au, Ag, Sm, and Ce, as well as all other rare earths commonly used to activate scintillators with the most preferred activating material being Ce (Cerium). According to the most preferred embodiment, the scintillating support bodies of the present invention consist of yttrium silicate glass activated with from about 0.1 to about 10.0 percent by weight of an inorganic cerium salt. Cerium is added to the yttrium silicate as an inorganic salt, preferably as the oxide, carbonate or chloride.

The scintillating support bodies of the present invention are most preferably formed as amorphous particles referred to for the purposes of this invention as beads. These beads are represented as spheres for the sake of clarity in FIGS. 1, 2 and 3. According to one possible preferred embodiment, cerium activated yttrium silicate glass is purchased from one of several manufacturers, or is prepared by methods well known in the art of glass manufacturing.

Glass particles in the range of from about 3 to about 100 microns in size are preferably utilized in the practice of the present invention. However, particles having a diameter of about 10 microns are most preferred in the practice of the present invention. According to one experiment, cerium-activated yttrium silicate glass beads of about 3, 10, and 100 microns in diameter were tested for counting efficiencies in SPRA. These trials were conducted using a liquid scintillation counter, ^{3}H, ^{14}C, and ^{32}P. In the trials utilizing the radiolabels ^{3}H and ^{14}C, particles having a 10-micron diameter yielded the highest counting efficiency. In the trials utilizing the radiolabel ^{32}P, the 3-micron particles yielded the greatest efficiencies. This data is summarized in FIG. 4.

The scintillating support bodies of the present invention, because they are constructed entirely from activated scintillating material, increase the accuracy of SPRA. Unlike other support bodies presently used in SPRA, which have scintillator scattered randomly throughout, alpha particles, beta particles, or auger electrons will not pass through the scintillating support body of the present invention without impacting and exciting the scintillating material. Accordingly, the frequency at which radioisotopes are in close proximity to the support body is more accurately detected and recorded.

The reactant of interest AG, the molecule for which the assay is being conducted to determine the concentration of, is represented in FIGS. 1 and 3 as elements 11 and 210,

respectively. AG is preferably selected from the group consisting of antibodies, antigens, deoxyribonucleic acids, ribonucleic acids, biological receptors, biological receptor antagonists, biological receptor agonists, steroids, enzymes, and vitamins. However, antigens or antibodies are the most preferred reactants of the present invention.

According to a preferred embodiment, the radiolabel is preferably selected from the group of radioisotopes consisting of ^{3}H, ^{125}I, ^{14}C, ^{131}I, ^{75}Se, ^{32}P and ^{57}Co. However, most preferably, the radiolabels are ^{3}H and ^{125}I. The radiolabelled reactant (AG*) is represented as elements 120 and 220 of FIGS. 2 and 3 respectively. AG* is preferably produced by chemically coupling AG to a radiolabel. It will be apparent to those skilled in this art that the methods and procedures for coupling radiolabels and reactants are well known and do not require further elaboration.

The radiolabel AG* emits either particulate or electromagnetic radiation. Most preferably, the radiolabel utilized in the practice of the present invention emits short range particulate radiation, i.e., alpha particles, beta particles, auger electrons or the like. The paths of these short range energies are indicated by arrows 130, 140 or 230, 240 of FIGS. 2 and 3, respectively. Arrows 130 and 230 represent alpha particles, beta particles or auger electrons whose paths terminate in the aqueous suspension without impacting and exciting the scintillating material of the scintillating support body. Arrows 140 and 240 represent the paths of alpha particles, beta particles, or auger electrons

which impact the scintillating material of the scintillating support body, thereby exciting the scintillator. The scintillator thereafter emits detectable photons of light, represented by arrows 150 or 250 of FIGS. 2 and 3, respectively. The emitted photons are received by a detector 190, 290, preferably the photomultiplier tube of a liquid scintillation counter.

The radiolabel of AG* is brought into close enough proximity to excite the scintillating support body by the ligands (AB) coupled to the surface of the support body, the ligands (AB) are represented as elements 16, 160 or 260 of FIG. 1, 2 and 3, respectively.

Ligands (AB) useful in the practice of the present invention indiscriminately and competitively biochemically bind to AG and AG*. The ligand is preferably selected from the group consisting of deoxyribonucleic acid, ribonucleic acid, biological receptor, biological receptor antagonist, biological receptor agonist, enzyme, vitamin, steroid and hormones.

Fig. 2 represents a direct assay of AG* 120. According to this preferred embodiment, the scintillating support body 100 is coupled with a multiplicity of AB 160, capable of selectively biochemically binding to AG*. AG* 120 is mixed with a plurality of scintillating support bodies 100, causing AG* to bind AB 160. The resulting complex, represented as element 170, brings the radiolabel of AG* 120 into close enough proximity to the scintillating support body to allow the radiation emitted 140 by the radiolabel, to excite the

scintillating support body, thereby causing the scintillating support body to emit photons of light 150. The photons 150 emitted are detected by a detector 190. From the detected photons of light a radioactivity level is determined. The radioactivity level measured is directly related to the formation of the AG*-AB complex 170. As the number of AG*-AB complexes 170 increases, the radioactivity level measured also increases proportionately, which in turn, is indicative of the amount of AG* 120 in the sample being assayed.

FIG. 3 represents a competitive assay system used to determine the quantity of AG 210 in a sample. According to one preferred embodiment, a known amount of AG* 220 and an unknown amount of AG 210 are added to an aqueous suspension containing a plurality of scintillating support bodies 200, each having a multiplicity of ligands (AB) 260 bound to its surface. AG* 220 and AG 210 thereafter compete to bind to AB 260. The reactants AG 210 and AG* 220 bind to AB 160 in proportion to their relative concentrations in the suspension, regardless of the presence of radiolabel. AG* 220 binds to AB 260 to form the complex AG*-AB 270. This brings the radiolabel of AG* 220 into close enough proximity to the scintillating support body 200 to cause the radiation emitted 240 by the radiolabel to excite the scintillating support body, thereby causing the scintillating support body to emit photons of light 250 which are detected by an outside detector 290. From the detected photons of light a radioactivity level is determined. In contrast to the direct assay method, the level of radioactivity measured is inversely proportional to

the amount of AG 210 in the sample. As the concentration of AG 210 increases so does the number of AG-AB 280 complexes, which in turn, results in a corresponding decrease in AG*-AB 270 complexes, and accordingly, a decrease in the level of radioactivity measured.

CLAIMS:

1. A reagent for use in scintillation proximity radioimmunoassay, comprising:

(a) a support body consisting of scintillating material; and

(b) the support body having coupled to its surface a multiplicity of ligands, said ligands being capable of selectivity biochemically binding to a reactant of interest.

2. The reagent of claim 1 wherein said scintillating material is insoluble in water.

3. The reagent of claim 1 wherein said scintillating material is one scintillator selected from the group consisting of an appropriately activated base glass and calcium fluoride.

4. The reagent of claim 1 wherein said scintillating material is activated yttrium silicate.

5. The reagent of claim 1 wherein said support body is formed into a bead.

6. The reagent of claim 5 wherein said bead has a diameter of from about 1 to about 300 microns.

7. The reagent of claim 5 wherein said bead has a diameter of about 10 microns.

8. The reagent of claim 4 wherein said activated yttrium silicate consists of yttrium silicate and an inorganic salt of one element selected from the group of elements consisting of Mn, Cu, Pb, Sn, Au, Ag, Sm, and Ce.

9. The reagent of claim 4 wherein said activated yttrium silicate consists of yttrium silicate and an inorganic salt of cerium.

10. The reagent of claim 8 wherein said activated yttrium silicate includes from about 0.1 to about 10.0% by weight of an inorganic salt of one said element.

11. The reagent of claim 9 wherein said activated yttrium silicate includes from about 0.1 to about 10.0% by weight of an inorganic salt of cerium.

12. A reagent for use in scintillation proximity radioimmunoassay, comprising:

(a) a support body consisting of yttrium silicate and from about 0.1 to about 10.0% by weight of an inorganic cerium salt; and

(b) the support body having coupled to its surface a multiplicity of ligands, said ligands capable of

selectively biochemically binding to a reactant of interest.

13. The process of assaying an aqueous suspension containing a known concentration of a radiolabelled reactant and an unknown concentration of non-radiolabelled reactant, said reactant being selected from the group consisting of antibodies, antigens, deoxyribonucleic acids, ribonucleic acids, biological receptors, biological receptor antagonist, biological receptor agonist, steroids, enzymes, hormones and vitamins, comprising:

a) adding to the suspension a plurality of scintillating support bodies consisting of scintillating material, the support bodies having coupled to their surface a multiplicity of ligands, said ligands being capable of selectively biochemically binding to said reactant, regardless of the presence of said radiolabel, said radiolabelled reactant emitting radiation energy capable of exciting a scintillating support body upon biochemically binding to a ligand, said scintillating support body producing detectable photons of light when excited; and

b) detecting the photons of light emitted by the plurality of scintillating support bodies in the aqueous suspension to determine the concentration of the non-radiolabelled reactant.

14. The process of claim 13 wherein said scintillating material is insoluble in water.

15. The process of claim 13 wherein said scintillating material is one scintillator selected from the group consisting of an appropriately activated base glass and calcium fluoride.

16. The process of claim 13 wherein said scintillating material is activated yttrium silicate.

17. The process of claim 13 wherein said scintillating support body is formed into a bead.

18. The process of claim 17 wherein said bead has a diameter of from about 1 to about 300 microns.

19. The process of claim 17 wherein said bead has a diameter of about 10 microns.

20. The process of claim 16 wherein said activated yttrium silicate consists of yttrium silicate and an inorganic salt of one element selected from the group of elements consisting of Mn, Cu, Pb, Sn, Au, Ag, Sm, and Ce.

21. The process of claim 16 wherein said activated yttrium silicate consists of yttrium silicate and an inorganic salt of cerium.

22. The process of claim 20 wherein said activated yttrium silicate includes from about 0.1 to about 10.0% by weight of an inorganic salt of one of said elements.

23. The process of claim 21 wherein said activated yttrium silicate includes from about 0.1 to about 10.0% by weight of an inorganic cerium salt.

24. The process of claim 13 wherein said photons of light emitted by the plurality of scintillating support bodies are detected by a commercially available liquid scintillation counter.

25. The process of claim 13 wherein the radiation energy emitted by the radiolibelled reactant is a beta particle.

26. A method of scintillation proximity radioimmunoassay, comprising:

a) providing a plurality of scintillating support bodies consisting of yttrium silicate and from about 0.1 to about 10.0% by weight of an inorganic salt of cerium;

b) coupling to the surface of said support bodies a multiplicity of ligands, said ligands being capable of selectively biochemically binding a reactant of interest, regardless whether it is radiolabelled;

c) providing a known quantity of the reactant chemically coupled to a radiolabel;

d) mixing in an aqueous suspension said known quantity of radiolabelled reactant and said support bodies;

e) adding an unknown quantity of non-radiolabelled reactant to said aqueous suspension, both non-radiolabelled and radiolabelled reactant specifically and competitively biochemically binding to said ligand, said radiolabeled reactant emitting radiation energy capable of exciting a support body upon biochemically binding to a ligand, said support body emitting detectable photons of light when excited; and

f) detecting the photons of light emitted by the plurality of scintillating support bodies in the aqueous suspension to determine the concentration of the non-radiolabelled reactant.

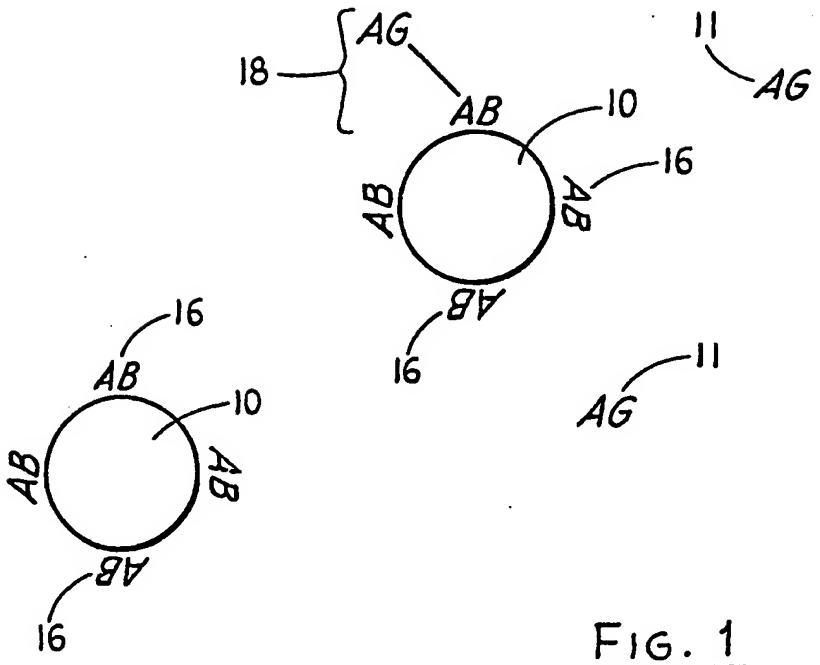

18
AG
AB
10
11
AG
16
AB
AB
AB
16
16
AB
10
AB
AB
AB
16
11
AG

FIG. 1

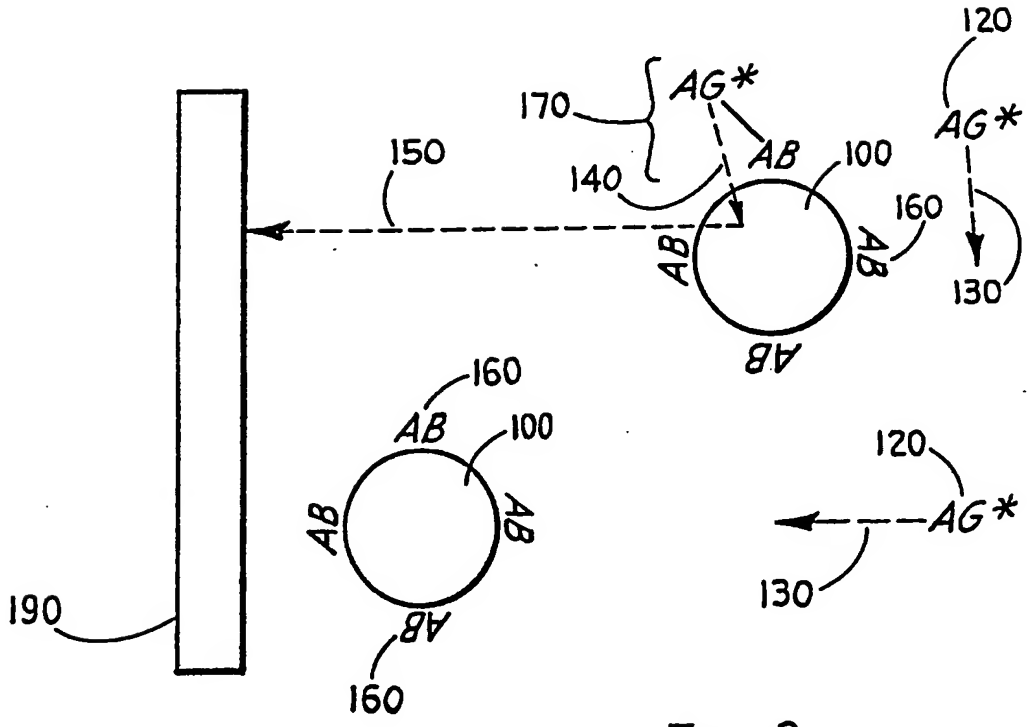

120
AG*
170
AG*
150
140
AB
100
160
AB
AB
AB
130
160
AB
100
AB
AB
190
120
AG*
130
160

FIG. 2

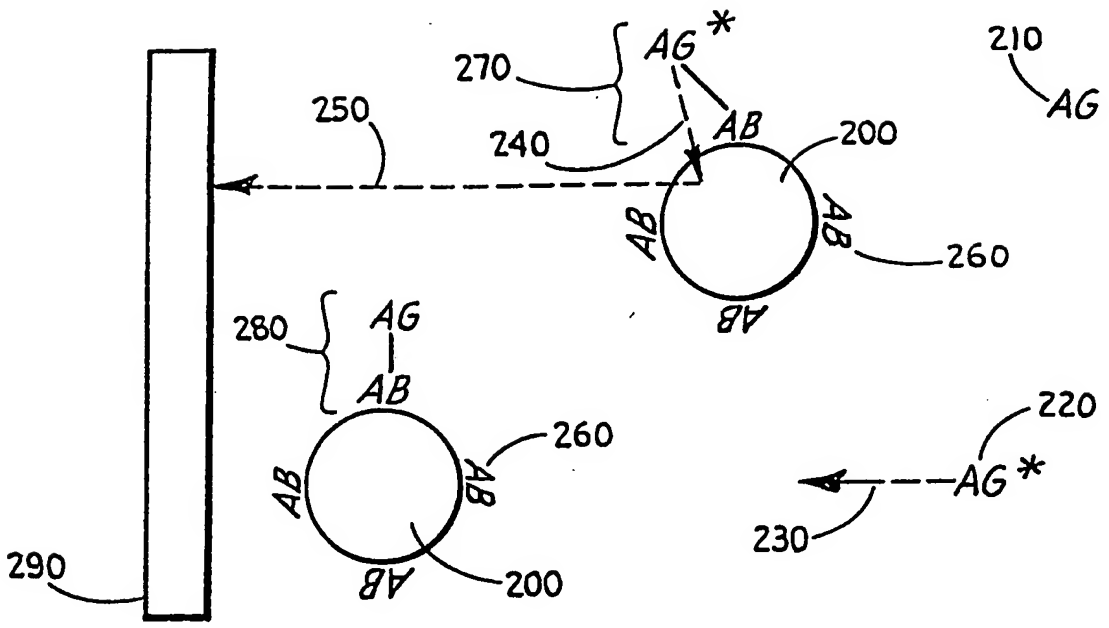

270
AG*
210
AG
250
240
AB
200
AB
AB
260
AB
280
AG
AB
260
AB
AB
220
AG*
230
290
200

FIG. 3

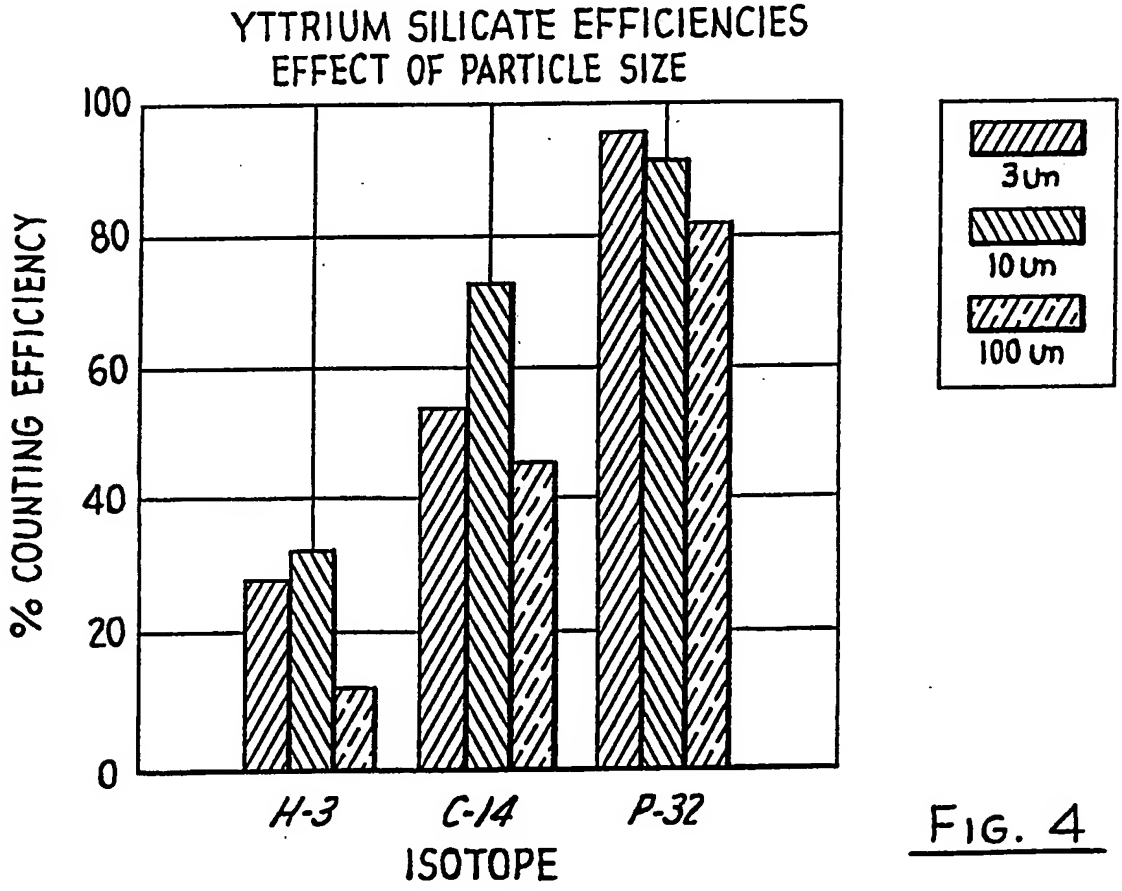

YTTRIUM SILICATE EFFICIENCIES
EFFECT OF PARTICLE SIZE
% COUNTING EFFICIENCY
100
80
60
40
20
0
H-3
C-14
P-32
ISOTOPE
3 Um
10 Um
100 Um

FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/06646

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): G01N 33/573; G01N 33/552; C12Q 1/68

U.S.Cl.: 435/6; 435/7.4; 436/527

II. FIELDS SEARCHED

Minimum Documentation Searched [4]

Classification System	Classification Symbols
U.S.Cl.	435/6; 435/7.4; 436/527; 436/518; 436/501; 436/804; 436/815; 436/808

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [5]

APS, CAS, search terms: Scintillation Proximity Assy, Yttrium Silicate, Ytriumsilicate and (Ceriumor CE ? or Calcium ORCA)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [14]

Category *	Citation of Document, [16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No. [18]
X, P Y	US,A, 4,916,320 (Wonderly et al.) 10 April 1990, See column 4, lines 15-27 and 58-64, column 5, lines 1-12.	1-9 10-26
Y	US,A, 4,568,649 (Bertoglio-Matte) 04 February 1986, See column 4, lines 7-27, column 8, lines 2-20, column 9, lines 27-30.	13-26
Y	US,A, 4,849,639 (Born et al.) 18 July 1989, See Abstract, column 1, lines 5-9, column 2, lines 25-26.	10-26

* Special categories of cited documents: [15]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search [2]	Date of Mailing of this International Search Report [2]
21 February 1991	21 MAR 1991
International Searching Authority [1]	Signature of Authorized Officer [20]
ISA/US	Susan C. Wolski Susan C. Wolski

Form PCT/ISA/210 (second sheet) (May 1986)